Title:	**Determinants of Common Carotid Intima-Media Thickness (CCIMT) Measured by Ultrasound Echo-tracking Method in Asymptomatic Individuals.**
Short Title	Determinants of CCIMT in Asymptomatic Individuals
Sponsor:	Dr. Srinivas Mantha, Principal Investigator(Self-funded)
Protocol Number	**MHC/CCIMT/001**
Version	01
Protocol Date:	30 November 2018

Principal Investigator
Dr. Srinivas Mantha, MD (Anesthesiology)
Consultant Anesthesiologist and Pain Physician
Indo-US Hospital, Ameerpet
Hyderabad 500016 (India)
And
Mantha's Pain Clinic, Barkatpura
Hyderabad 500027 (India)

Funding: Self-funded by Dr. Srinivas Mantha

Protocol for submission to **Institute Ethics Committee of Indo-US Hospital, 7-1-57/B&C, Shyam Karan Road, Ameerpet, Anand Bagh, Hyderabad, Telangana 500016**

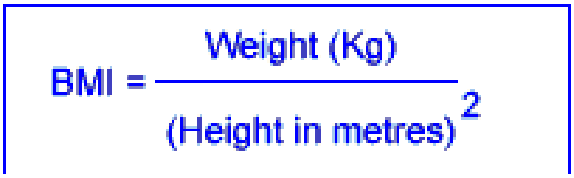
Co-Investigaors
Dr. T. Sudha Lakshmi, MD, DM (Cardiology)
Consultant Cardiologist
Mantha Heart Clinic
Barkatpura
Hyderabad 500027 (India)
drsudha65@yahoo.co.in

Dr. Dasari Prasada Rao, MS, MCh (Cardiothoracic Surgery)
Consultant Cardiothoracic Surgery
Indo-US Hospital
7-1-57/B&C, Shyam Karan Road, Ameerpet, Anand Bagh
Hyderabad 500016 (India)
prasadarao.dasari@rediffmail.com

Table of Contents

A. ABBREVIATIONS AND DEFINITIONS OF TERMS

Term/Abbreviation	Definition / Full form
Arteries	Arteries are blood vessels that carry oxygenated blood from the heart to entire body.
Artery layer Intima-media	In practice, inner wall thickness is referred to as 'intima-media', i.e. comprised of both tunica-intima and tunica media
Artery layer: Endothelium	A thin innermost layer of cells is called the endothelium. The endothelium works to keep the inside of arteries toned and smooth, which keeps blood flowing.
Artery layer: Tunica adventitia	Connective tissue that surrounds an artery
Artery layer: Tunica intima	It is made up of one layer of endothelial cells and is supported by an internal elastic lamina.
Artery layer: Tunica media	Middle layer of an artery. It is made up of smooth muscle cells and elastic tissue. It lies between tunica intima on the inside and tunica adventitia on the outside
Artery wall (Layers)	Composed of innermost (exposed to blood flow) to outer are endothelium, tunica intima, tunica media, tunica adventitia
ASA Physical status grading	American Society of Anesthesiologists (ASA) physical status grading ASA PS I: Healthy individual ASA PS II: Mild diseases only without substantive functional limitations and well controlled diabetes or hypertension ASA PS III: Severe systemic disease with Substantive functional limitations, e.g. Poorly controlled diabetes or hypertension ASA PS IV: A patient with severe systemic disease that is a constant threat to life, e.g. recent heart attack, stroke ASA PS V: A moribund patient ASA PS VI: Declared brain dead patient ASA grade I and grade II are considered healthy otherwise
Asymptomatic	In medicine, a disease is considered asymptomatic if a patient does not

<table>
<tr><th>Term/Abbreviation</th><th>Definition / Full form</th></tr>
<tr><td></td><td>experience symptoms in others words ‘showing no evidence of disease’;</td></tr>
<tr><td>Atherosclerosis</td><td>Hardening and silent narrowing of the arteries which results in reduced blood flow
It’s the usual cause of heart attacks, strokes, and peripheral vascular disease -- what together are called cardiovascular disease.</td></tr>
<tr><td>BMI</td><td>Body mass index is one of the ways to determine when extra fat accumulation translates into health risks
 
$$BMI = \frac{\text{Weight (Kg)}}{(\text{Height in metres})^2}$$
For example, a person weighing 60 kg having a height of 160 cm will have BMI of 23.4
<table><tr><td>18.5 to 24.9</td><td>25.0 to 29.9</td><td>30.0 to 39.9</td><td>>40</td></tr><tr><td>Normal Weight</td><td>Overweight</td><td>Obesity</td><td>Morbid Obesity</td></tr></table></td></tr>
<tr><td>Cardiac Risk Stratification</td><td><table><tr><th>Z score</th><th>Percentile</th><th>Cardiac Risk Stratification*</th></tr><tr><td>< -0.675</td><td>< 25th</td><td>lower than the expected</td></tr><tr><td>-0.625 to <0.625</td><td>25th to 74th</td><td>Unchanged risk</td></tr><tr><td>≥ 0.625 to <1.960</td><td>75th to 97.5</td><td>Increased risk</td></tr><tr><td>≥ 1.960</td><td>≥ 97.5th</td><td>Very high risk</td></tr></table>*Risk compared individuals of equal age and gender</td></tr>
<tr><td>CCIMT</td><td>Common Carotid Intima-media Thickness. In medical practice CCIMT is measured by ultrasound by placing the probe over the neck on either side to measure the value on either side. Standard unit is µm (1mm=1000 µm)</td></tr>
<tr><td>Common Carotid Artery</td><td>Artery that is present in the neck and supplies blood to brain and other parts of the head.</td></tr>
<tr><td>Multiple Linear Regression Analysis (MLR)</td><td>MLR examines how multiple independent variables are related to one dependent variable. Once each of the independent factors have been determined to predict the dependent variable, the information on the</td></tr>
</table>

Term/Abbreviation	Definition / Full form
	multiple variables can be used to create an accurate prediction on the level of effect they have on the outcome variable. The model creates a relationship in the form of a straight line (linear) that best approximates all the individual data points. In the present context, dependent variable will be CCIMT (or its derivatives) and independent variables will be (smoking status (yes/no), diabetes, (yes/no), BMI, WhtR, TC-HDL ratio, blood vitamin D3 levels) etc.
Percentile	Percentile usually indicates that a certain percentage falls below that percentile. For example, if a measurement value is in the 75th percentile, then 75% individuals will have the value are below that is observed. In the context of CCIMT measurement, higher the percentile the more will be the thickness and therefore suggests higher risk.
Plaque	Plaque creates a bump on the artery wall. As atherosclerosis progresses, that bump gets bigger. When it gets big enough, it can create a blockage of blood flow resulting in heart attack, stroke etc
Plaque rupture	Plaques can suddenly rupture, allowing blood to clot inside an artery. In the brain, this causes a stroke; in the heart, a heart attack.
Screening	Medical screening is the systematic application of a test or inquiry to identify individuals at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action
Surrogate marker	A laboratory measurement or physical sign that is used as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy.
TC-HDL Ratio	Total cholesterol (TC) in the human body consists of low-density lipoprotein (LDL, or “bad”) cholesterol and high-density lipoprotein (HDL or “good”) cholesterol. Cholesterol is a waxy, fat-like substance found in every cell in your body.TC-HDL ratio is a number is obtained by dividing total cholesterol by HDL (good) cholesterol. For example, if a person has a total cholesterol of 200 mg/dL and an HDL cholesterol level of 50

Term/Abbreviation	Definition / Full form
	mg/dL, the ratio would be 4.0. A high ratio indicates a higher risk of cardiovascular events while a low ratio indicates a lower risk.
Ultrasound machine	Also known as diagnostic sonography or ultrasonography. is a diagnostic imaging technique based on the application of ultrasound (sound waves). It is used to see internal body structures such as tendons, muscles, joints, blood vessels, and internal organs.
Ultrasound probe	A part that is placed on the human body intended to send and receive sound signals for processing by the ultrasound machine
Vascular Age	It is another way to quantify the atherosclerotic burden from CCIMT by comparing an individual's IMT against the mean CCIMT (50^{th} percentile) in the standard age-IMT table. For example, if a woman aged 40 years has a CCIMT value of 709 micrometers, then the vascular age would be 78 years as the CCIMT value corresponds that of a woman aged 78 year as per the standard population
Waist-to-height ratio (WHtR)	Another anthropometric measurement ratio waist circumference at just above umbilicus level and height with both measured in the same units. For example, an individual with waist circumference of 100 cm and a height of 175 cm will have WHR of 0.57. It is measure of distribution of body fat and higher values indicate higher risk of obesity-related cardiovascular diseases. It is correlated to abdominal obesity. A WHtR of over 0.5 is critical and signifies an increased risk.
Z score	As CCIMT increases progressively with age (Appendix 1) and absolute IMT value may be difficult to interpret in a given individual. Hence a better metric in such cases would be z score, the general formula for which is $z = \frac{x - \mu}{\sigma}$ $\mu =$ Mean $\sigma =$ Standard Deviation X = Observed value In the context of this protocol z score will be computed as

<table>
<tr><th>Term/Abbreviation</th><th>Definition / Full form</th></tr>
<tr><td></td><td>$\frac{\text{(observed IMT- expected IMT for age and gender)}}{\text{SD IMT for that age and gender}}$</td></tr>
<tr><td>z score and percentile</td><td>
<table>
<tr><th>Z</th><th>Percentile</th></tr>
<tr><td>-1.960</td><td>2.5th</td></tr>
<tr><td>-0.675</td><td>25th</td></tr>
<tr><td>0</td><td>50th</td></tr>
<tr><td>0.675</td><td>75th</td></tr>
<tr><td>1.960</td><td>97.5th</td></tr>
</table>
</td></tr>
</table>

B. ABSTRACT

Background:

Common carotid artery intima-media thickness (CCIMT) is a surrogate marker for atherosclerosis which starts early in life and progresses slowly from asymptomatic stage to symptomatic stage with manifestations such as heart attacks, strokes, kidney disease and peripheral vascular disease. CCIMT is measured by ultrasound and an accurate method called 'echo-tracking' is now available. The method relies on automated edge detection by radiofrequency signal processing of ultrasound. Research question in hand is whether CCIMT can be predicted from readily available clinical variables.

Objectives: (**Primary and important secondary objectives)**

The main objective is to measure CCIMT (dependent variable) in other-wise healthy asymptomatic individuals and examine whether any or combination of the following variables (independent clinical variables) can predict it: gender, chronological age, body mass index (BMI), Waist-to-Height Ratio (WHtR), cigarette smoking status (yes/no), diabetes status (yes/no), TC-HDL ratio, blood vitamin D3 levels. Secondary objective is to determine proportion of individuals who will be categorized as 'very high risk' by CCIMT measurement These individuals require immediate attention for further evaluation and management.

Study Design:

Observational study with single arm

Setting/Participants:

Outpatient clinic in 122 asymptomatic and otherwise healthy individuals of either gender aged between 20 and 60 years. They can be volunteers or those consulting for wellness preventive care. Individuals with known cardiovascular disease and those taking lipid lowering drugs or who have taken treatment for vitamin D deficiency will be excluded. The study centers will be either Indo-US Hospital, Ameerpet, Hyderabad 500016, Telangana, India or Mantha Heart Clinic, Barkatpura, Hyderabad 500027, Telangana, India,

Study Measures: Main study measure would be CCIMT measured by B-mode ultrasonography using 3-13 MHz linear probe. An accurate method called 'echo-tracking' that relies on automated edge detection by radiofrequency signal processing of ultrasound will be used. The ultrasound machine will be MyLab Gamma Portable machine of Esaote make. To develop a mathematical model (linear regression analysis) the

following anthropometric measurements (BMI and WHR) will be made and a blood sample of about 5 ml will be taken for blood tests related to lipid profile, blood sugar, creatinine and vitamin D3)

Analysis for Results: Since CCIMT increases proportionately with age, absolute CCIMT observed in a patient will be difficult to interpret. Hence derivates of CCIMT that quantify atherosclerotic burden i.e. z score and vascular age will be used as applicable. A multiple linear regression analysis will be used to identify determinants (independent clinical variables) stated above that can predict CCIMT z score through a mathematical model.

C. PROTOCOL SYNOPSIS

Study Title	**Determinants of Common Carotid Intima-Media Thickness (CCIMT) Measured by Ultrasound Echo-tracking Method in Asymptomatic Individuals.**
Funder	Dr. Srinivas Mantha, Principal Investigator (Self-funded)
Study Rationale	• Atherosclerosis implies silent hardening and narrowing of the arteries resulting in obstruction to blood flow. Usual progression of the disease is slow from asymptomatic stage to symptomatic stage with manifestations such as heart attacks, strokes, kidney disease and peripheral vascular disease. • Ultrasound is a noninvasive diagnostic imaging technique based on the application of ultrasound (sound waves). It is used to see internal body structures such as tendons, muscles, joints, blood vessels, and internal organs. • Common carotid artery intima-media thickness (CCIMT) is a surrogate marker for atherosclerosis which starts early in life and CCIMT is measured by ultrasound and an accurate method called 'echo-tracking' is now available. The method relies on automated edge detection by radiofrequency signal processing of ultrasound. • The CCIMT measurement is done by ultrasound machine by placing a probe over the neck on either side. The measurement is in routine radiology practice during ultrasound cerebrovascular studies for over more than 2 decades. Usually, the test is completed within 15 minutes. • Research question in hand is whether CCIMT can be predicted from readily available clinical variables in healthy asymptomatic individuals. • Rationale is to develop a mathematical model (equation) to predict

	CCIMT from readily available clinical data (physical examination and routine laboratory test results) so that cardiac risk stratification can be done in day-to-day practice without use of expensive ultrasound machine.
Study Objective(s)	**Primary** • The main objective is to measure CCIMT in other-wise healthy asymptomatic individuals and examine whether any or combination of the following variablescan predict it: gender, chronological age, body mass index (BMI), Waist-to-Height Ratio (WHtR), cigarette smoking status (yes/no), diabetes status (yes/no), TC-HDL ratio, blood vitamin D3 levels. To develop a mathematical equation to predict CCIMT from the variables tested to be significantly associated. **Secondary** • Secondary objective is to determine proportion of individuals who will be categorized as 'very high risk' from CCIMT measurement. These individuals require immediate attention for further evaluation and management.
Study Design	Observational study with single arm.
Subject Population **key criteria for Inclusion and Exclusion:**	**Inclusion Criteria** • Asymptomatic and otherwise healthy individuals of either gender aged between 20 and 60 years (volunteers or those attending outpatient wellness clinic for preventive care • Functionally independent with well controlled mild systemic disease (e.g. controlled hypertension or diabetes) **Exclusion Criteria** • Individuals with known cardiovascular disease • Taking lipid lowering drugs • Who have taken treatment for vitamin D deficiency

Study Duration	• Each subject's participation will last about an hour • The entire study is expected to last about one year
Study Phases **Screening** **Study Treatment** **Follow-Up**	• Screening: screening for eligibility and obtaining consent • Observation Period: measurements made for measured CCIMT by ultrasound and taking blood sample (about 5ml) for routine blood tests
Efficacy Evaluations	CCIMT measurement by ultrasound to help cardiac risk stratification
Safety Evaluations	• Although not routinely practiced in day-today in cerebrovascular studies by radiologists, pulse rate and oxygen saturation will be monitored during the ultrasound study of CCIMT • Individuals who will be categorized as 'very high risk' from CCIMT measurement will be referred to regular cardiology clinics for further evaluation
Statistical And Analytic Plan	• CCIMT increases proportionately with age, absolute CCIMT observed in a patient will be difficult to interpret. • Hence derivates of CCIMT that quantify atherosclerotic burden i.e. z score and vascular age will be used as applicable. • A multiple linear regression analysis will be used to identify determinants (independent clinical variables) stated above that can predict CCIMT z score through a mathematical model.
DATA AND SAFETY MONITORING PLAN	• The primary investigator will be responsible for data management and patient safety • Indo-US hospital (Site 1) will monitor the data acquisition and store the case report forms (CRF) related to the study

1. Background Information and Rationale

Atherosclerosis is the principal cause of adverse cardiovascular events. Usual progression of the disease is slow from asymptomatic stage to symptomatic stage with manifestations such as heart attacks, strokes, kidney disease and peripheral vascular disease. Carotid intima-media thickness (CCIMT) is increasingly used as a surrogate marker for atherosclerosis. (1). CCIMT is routinely measured as part of noninvasive cerebrovascular studies by ultrasound in diagnostic centers. The intima-media thickness (IMT) represents the thickness of tunica intima and tunica media, the innermost two layers of the wall of an artery. Although commonly measured in the common carotid arteries, it quantifies atherosclerotic burden in entire vascular tree including that in the heart. Hence, it is used in cardiac risk stratification and predict future clinical cardiovascular events. Since it is neither practical nor recommended to refer asymptomatic individuals for cerebrovascular ultrasound studies, prediction of CCIMT from readily available clinical information would useful.

The aim of this proposed study is to identify clinical and common blood test variables that determine the CCIMT and develop a model (mathematical equation) to determine CCIMT from such identified readily available variables in routine practice, in otherwise healthy individuals, without need for ultrasound machine. Practical use of results of this study to estimate CCIMT from readily available clinical variables in day-to-day practice without need for diagnostic cerebrovascular studies by ultrasound.

1.1 Introduction

An observational study in asymptomatic or otherwise healthy individuals in outpatient setting is planned to study the clinical determinants of CCIMT obtained by ultrasound examination of carotid arteries in the neck. The subject enrolled in the study could be volunteers or those attending wellness clinics for preventive care. The determinants, if identified from this study, will permit us to estimate CCIMT without need for actual use of ultrasound examination. The study intends to develop a mathematical model to predict CCIMT. Such model will be helpful for doctors in routine clinical practice to do cardiac risk stratification without need for ultrasound studies. High risk cases thus identified can be referred to cardiologists for further evaluation. Another potential use of such models is in preoperative cardiac risk stratification for non-cardiac surgery. The preoperative risk stratification algorithms typically use a Bayesian approach to identify a low-risk category group who can go for surgery without further testing.

1.2 Relevant Literature and Data

Atherosclerosis is a disease in which inside of the artery narrows due to buildup of plaque. Plaque is made up of cholesterol, calcium, and other substances found in the blood. Atherosclerosis is present early in life and may be asymptomatic for a long before the occurrence of cardiovascular events such as heart attack, stroke, kidney diseases etc. Risk factors for atherosclerosis include abnormal blood cholesterol levels, diabetes, smoking, obesity, family history of cardiovascular disease, unhealthy diet and sedentary life style. Progression from asymptomatic stage to occurrence of symptoms usually depends on the number of risk factors and individuals with genetic predisposition for vascular disease.

There has been mounting evidence linking vitamin D deficiency to cardiovascular disease and atherosclerosis. Vitamin D deficiency affects almost 50% of the population worldwide. It has been suggested that this pandemic might contribute to the worldwide increased prevalence of cardiovascular disease (CVD). Vitamin D exerts protective effects on atherosclerosis through multiple mechanisms including protecting against endothelial dysfunction, dyslipidemia and insulin resistance.(2) Recently, it was shown that treatment with vitamin D3 can significantly restore the damage to the cardiovascular system, while also reducing the risk of heart attack. (3). A retrospective study that involved 3509 patients found that decreased Vitamin D concentrations in blood were associated with a composite of in-hospital death, serious infections, and serious cardiovascular events after noncardiac surgery(4). An editorial accompanying the article, suggested prospective studies to ascertain the facts related to such association and interventions required for appropriate management (5)

Early detection of atherosclerosis has a potential to predict the risk of future cardiovascular events. This will permit management of atherosclerosis with life style changes, diet modification and medications as part of wellness care in prevention or limiting the progression of atherosclerosis. In both men and women there is a linear relationship between age and CCIMT (6). CCIMT is measured by B-mode ultrasonography. To circumvent the problems related to variability of measurement of IMT, a new method called 'echo-tracking' is now available in modern ultrasound machines for accurate measurements. The method relies on automated edge detection by radiofrequency signal processing of ultrasound.

The unit of measurement of IMT is μm (micrometers) and 1 millimeter (mm) = 1000 μm. In both men and women, the IMT increases progressively with age, e.g. 5.2 μm/year in men and 5.0 μm/year in women. (6).

At the age of 15 years it is about 402 µm in boys and about 396 µm in girls increasing to about 688 µm and 670 µm men and women respectively when to age to 70 years.

In healthy sub-population aged above 15 years, the IMT (in micrometers) is estimated by the following formulas.

In men, mean = 323.5 + (5.201 × age), SD = 57.24 + (0.9027 × age)

In women, mean = 321.7 + (4.971 × age), SD = 54.50 + (0.8256 × age).

Hence, because of availability of mean and SD at each age point, percentiles can be estimated by applying standard formulas. Based on the observed CCIMT value, two variables from clinical management standpoint may be derived, namely z score and vascular age. Both variables can reflect the extent of abnormality related to atherosclerosis and estimate future cardiovascular risk.

Z Score

As CCIMT increases progressively with age (Appendix 1) and absolute IMT value may be difficult to interpret in a given individual. Hence a better metric in such cases would be z score, the general formula for which is

$$z = \frac{x - \mu}{\sigma}$$

$\mu =$ Mean
$\sigma =$ Standard Deviation

X = Observed value

In the present context, the IMT_Z score will be computed as

$$\frac{(\text{observed IMT- expected IMT for age and gender})}{\text{SD IMT for that age and gender}}$$

Z score values may be converted to percentile values by the following table.

Z	Percentile
-2.326	1st
-1.960	2.5th
-1.645	5th
-1.282	10th

-0.675	25th
0	50th
0.675	75th
1.282	90th
1.645	95th
1.960	97.5th
2.326	99th

A z score value -0.625 for IMT is equivalent to 25th percentile which implies that 75% of individuals of the same age and gender will have higher IMT value than that observed in the individual. A z score value 1.282 for IMT is equivalent to 90th percentile which implies that 10% of individuals of the same age and gender will have higher IMT value than that observed in the individual. In context of IMT measurement, higher the z scores and hence the higher percentiles will represent more abnormality.

Z score	**Percentile**	**Implication with regard to cardiovascular risk**
< -0.625	< 25th percentile	lower than the expected cardiovascular risk.
≤ -0.625 to <0.625	25th to 74th Percentile	unchanged cardiovascular risk
≥ 0.625 to <1.960	75th to 97.5 Percentile	increased cardiovascular risk
≥ 1.960	> 97.5 Percentile	Very high risk of cardiovascular events such as heart attack and stroke and requires immediate attention

It is speculated that percentile value between 75 and 97.5 may require life-style modification (walking to cover about 10,000 steps a day, exposure to morning sun light, diet modifications to reduce intake of saturated fats etc) whereas value above 97.5 percentile may require additional laboratory tests (blood sugar, serum creatinine, lipid profile and vitamin D3) and medications as per requirement. Medications typically include statins, aspirin from preventive standpoint in addition to other medications to control blood pressure and diabetes as per findings in a patient. Vitamin D3 therapy will be required if such deficiency is documented by laboratory testing.

For example, let us assume a male aged 40 years has CCIMT value of 636 µm (0.636 mm) found by ultrasound examination as described. Expected IMT values for mean (or 50th Percentile) and standard

deviation (SD) respectively are 532 µm and 93 respectively for 40-year old male individuals. Hence the z score would be (636-532)/93 = 1.112.

Vascular Age:

Availability of nomograms for CCIMT in different groups (age, gender, race etc.) permits one to determine the '*vascular age'* as opposed to the individual's chronological age. (6,7),(8). Chronological age of an individual may be adjusted based on atherosclerotic burden as evaluated by CCIMT. The CCIMT value higher than expected for similar age and gender implies higher vascular age. The vascular age may be quantified by comparing an individual's IMT against the mean CCIMT (50^{th} percentile) in the standard age-IMT table. For example, if a woman aged 40 years has a CCIMT value of 709 micrometers, then the vascular age would be 78 years as the CCIMT value corresponds that of a healthy womanaged 78 years.

Practical Application of Information from CCIMT in Clinical Practice

Wellness Clinics

Healthy individuals or those who are otherwise considered normal except for other health issues (hypertension controlled with medications, family history of early onset cardiovascular disease, vitamin D deficiency etc.) usually approach wellness clinics for guidance and preventive management to limit future risk of cardiovascular events. Information from CCIMT will be useful in this regard.

Preoperative Cardiac Risk Stratification for Non-cardiac Surgery

Postoperative mortality remains a serious problem after noncardiac surgery with major cardiovascular events contributing to about one-third of all perioperative deaths (9). Both cardiac and noncardiac adverse events have a major impact long term disability and mortality after non-cardiac surgery. Myocardial injury documented by postoperative troponin elevation is associated with increased risk of death and disability (10). Preoperative cardiac risk stratification algorithms aim to reduce postoperative morbidity and mortality. They typically use a Bayesian approach to identify a low-risk category group who can go for surgery without further testing. Bayes' theorem states that the probability of an event is based on previous knowledge of conditions that might be related to the event. A recent study found that three popular prediction models disagreed 29% of the time by which patients were categorized as low risk (<1%) (11). Hence, an approach to strengthen and optimize the Bayesian risk indices is needed (12). However, in preoperative cardiac risk stratification, information from CCIMT appears to be useful as part of shared decision making to determine if

long-term survival is important with respect to the decision to operate. Preoperative statin therapy and close monitoring with troponin may be considered to reduce immediate perioperative risk.

1.3 Compliance Statement

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with the standard regulations related to conduct of research in human subjects. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2. Study Objectives

Primary Objective: The main objective is to measure CCIMT (dependent variable) in other-wise healthy asymptomatic individuals and examine whether any or combination of the following variables(independent clinical variables) can predict it: gender, chronological age, body mass index (BMI), Waist-to-Height Ratio (WHtR), cigarette smoking status (yes/no), diabetes status (yes/no), TC-HDL ratio, blood vitamin D3 levels.

Secondary objective is to determine proportion of individuals who will be categorized as 'very high risk' from CCIMT measurement. These individuals require immediate attention for further evaluation and management.

3. Investigational plan

3.1 General Schema of Study Design

A prospective observational single arm study in healthy individuals.

3.2 Study Duration, Enrollment and Number of Sites

Time required for each subject including the screening procedure for enrolment is about one hour

Total Number of Subjects: (122)

Total Number of Sites (2)

- Site 1: Indo-US hospital, Ameerpet, Hyderabad
- Site 2: Mantha Heart Clinic, Barkatpura, Hyderabad

Inclusion Criteria

1. Asymptomatic and otherwise healthy individuals of either gender aged between 20 and 60 years. The subjects can be volunteers or those attending outpatient wellness clinic for preventive care
2. Functionally independent with well controlled mild systemic disease (e.g. controlled hypertension or diabetes). The group typically refers to ASA physical status grade I and II should they present for preoperative evaluation for noncardiac surgery
3. Can walk one km or 30 min walking or can climb more than 2 flights of stairs

Exclusion Criteria (At screening stage)

1. Individuals with known cardiovascular disease
2. Newly diagnosed without treatment or uncontrolled diabetes
3. Serum creatinine >2 mg% or known renal failure
4. Taking lipid lowering drugs
5. Taking or Who have taken treatment for vitamin D deficiency

Exclusion Criteria (Post-enrolment)

1. Newly diagnosed without treatment or uncontrolled diabetes
2. Technical errors in capturing ultrasound images of carotid arteries precluding proper assessment of CCIMT
3. Time beyond 30 minutes in acquiring proper ultrasound images of carotid arteries suitable for proper assessment of CCIMT
4. Serum creatinine >2 mg%

4 Study Procedures:

After screening involving medical history and clinical examination informed consent will be taken in eligible subjects followed by measurements and tests specific to the protocol as follows:

A. **Anthropometric measurements**, weight, height, waist circumference just above umbilicus

B. **Sample for blood tests**: 5ml of blood will be drawn for the following blood tests

1. Random blood sugar
2. Serum creatinine
3. Lipid profile
4. Vitamin D3

5. **Ultrasound scan for CCIMT measurement**

5. Study Evaluations and Measurements

As the CCIMT measurement by ultrasound is the key variable in this study, the same will be described in detail.

a. Main study measure would be CCIMT measured by B-mode ultrasonography using 3-13 MHz linear probe.
b. The ultrasound machine will be MyLab Gamma Portable machine of Esaote make.
c. An accurate method called 'echo-tracking' that relies on automated edge detection by radiofrequency signal processing of ultrasound will be used. The Esaote company named the method as 'QIMT' for quality intima-media thickness.
d. Vascular mode will be selected for examination
e. The length for region of interest (ROI) is 1.5 cm and will be fixed in the machine as default for all the cases.
f. Patient will then be taken to scan room and made to lie on the bed with head turned to opposite side to that being examined. A finger pulse oximeter will be placed to monitor heart rate and oxygen saturation.
g. CCIMT will be measured on right side first then the left side.
h. For each side, the probe will be placed on the neck in transverse position to obtain short axis view of carotid artery which can be seen as pulsating vessel on the screen. The probe will be moved to acquire the artery view in the middle of the screen. Then the probe will be changed to vertical direction to get the long axis view of carotid artery. The button for 'QIMT' will be pressed. The probe position is adjusted to ensure that green vertical line is just proximal to the carotid bulb. This ensures that CCIMT measurement commences from about 1 cm from carotid bifurcation and extends 1.5 cm beyond.
i. During scanning for QIMT, average of six successive measurements with standard deviation less than 10 will be taken, provided dense green overlay is fully displayed along the full common carotid artery borders lying inside the ROI of 1.5 cm.
j. The images and data are then stored in the ultrasound machine and transferred to a computer.
k. Three such measurements of CCIMT for each side are made and one best measurement is taken for that side. Higher of the two corresponding to CCIMT of right and left are taken for calculation of z score and vascular age.

l. If the ideal images with data required for proper CCIMT measurement are not obtained even after 15 minutes, then the scanning will be tried in the sitting position. In any case, scanning will not be done beyond 30 minutes. In case of failure to acquire the images and data within that time, the procedure will be abandoned and the patient will be excluded from the study.

m. All scans will be done by a single doctor, SM, the primary investigator of the study who is experienced in QIMT measured on EsaoteMylab Gamma machine.

n. More technical details of the machine, image and data acquisition are presented in the investigator's brochure.

o. Ideal image and data are depicted in the figure below

Total Time taken for study from screening to end of scanning will be about one hour

6. Statistical Considerations

6.1 Selection of Variables to be Tested in the Model

In any prospective study that intends to use multivariate regression analysis in the prediction model, selection of variables to be tested is very important as sample size estimation is critically dependent on variables to be tested. CCIMT measurement with an accurate method such as 'echo-tracking' that relies on automated edge detection by radiofrequency signal processing of ultrasound is available only with modern expensive machines. Hence, there is a need to identify clinical and common blood

test result variables that determine the CCIMT and develop a model (mathematical equation) to determine CCIMT from such identified variables applicable in routine practice. CCIMT increases proportionately with age, absolute CCIMT observed in a patient will be difficult to interpret. Hence derivates of CCIMT that quantify atherosclerotic burden i.e. z score and vascular age will be used as applicable.

A study published in 2013 involving systematic review of literature, identified the following variables to be associated increased CCIMT in healthy individuals: age, diabetic status, smoking history status, systolic BP, TC-HDL ratio.(6) The study developed independent models in either gender. From a clinical series of 44 cases, 22 were segregated who would otherwise be regarded as healthy in wellness clinics. Analysis was made from these individuals who came for wellness care at the clinic of first and second author of this protocol (SM & TSL). From a preoperative evaluation perspective, they would qualify for low-risk category should they present for noncardiac surgery. However, 4 individuals out of 24 were stratified as 'very high risk' i.e. CCIMT measurement z score ≥1.96 (≥95th percentile). They were started on medications as per current guidelines. The rationale of the retrospective analysis was to get inputs for sample size estimation for the proposed study. Multivariate linear regression analysis was done with CCIMT z score as dependent variable and following as independent variables: body mass index (BMI), waist-to-height ratio (WHR), total cholesterol HDL ratio (TC-HDL ratio) and serum vitamin D3 levels. TC-HDL ratio was found to be significantly associated with CCIMT z score. In addition, vitamin D3 also appeared to be closely related, but not significantly to CCIMT z score. Based on the above information, the following variables would be included as independent variables in the present study: age, gender, cigarette smoking status, diabetic status, body mass index (BMI), waist-to-height ratio (WHR), total cholesterol HDL ratio (TC-HDL ratio), serum vitamin D3 to test for their possible association with CCIMT z score.

6.2 Sample Size Estimation

The sample size was calculated with PASS (Power Analysis and Sample Size) software PASS 16 Power Analysis and Sample Size Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass with inputs as described below

Regression Model type	Power	Alpha error	Independent variables controlled	Independent variables tested	Effect size

Unconditional (Random X's)	0.8	0.05	0	8	0.15

With these assumptions, the sample size for the proposed study would be 111 and additional 11 individuals i.e. 10% of the estimated sample size will be recruited to account for loss of data resulting from technical errors in measurements or inability to capture the ultrasound images for correct CCIMT measurement. Hence, the study is proposed to enroll in 122 subjects.

After completion of study, the data will be compiled for analysis. The data analysis will be made using NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss. Initial analysis employing multivariate linear regression analysis will be done using CCIMT z score as dependent variable and the following eight variables as independent variables: age, gender, smoking status, diabetic status, BMI, WHtR, TC_HDL ratio and vitamin D3 levels. The variables that are statistically significantly associated with CCIMT z score ($p < 0.05$) will be selected. If only one variable is found to be significant, then a simple linear regression analysis will done to get the prediction model otherwise multiple linear regression analysis will be done on the short-listed variables.

7. Safety Management:

Although not routinely practiced in day-to-day in cerebrovascular studies in diagnostic centers, pulse rate and oxygen saturation will be monitored during the ultrasound study of CCIMT.Individuals who will be categorized as ‘very high risk’ from CCIMT measurement will be referred to regular cardiology clinics for further evaluation. Lesser risk category cases will be counselled or referred as per current clinical guidelines.

7.1 Clinical Adverse Events:

Despite the fact the CCIMT measurement by ultrasound is noninvasive and safe, clinical adverse events (AEs) will be monitored throughout the study

7.2 Adverse Event Reporting

The primary investigator (SM) will be responsible for recording and reporting unanticipated problems related to research that occur during and after study treatment.

8. Study Administration:

Patients will not be charged for anything related to the study i.e. consultation, blood tests, CCIMT measurement. However, subjects will not be reimbursed for travel and meals etc.

The primary investigator will be responsible for data management and patient safety. Indo-US hospital (Site 1) will monitor the data acquisition and store the source documents and case report forms (CRF) related to the study. Complete confidentiality of data will be maintained.

9. Contribution of Investigators and Authors for Potential Publication:

Investigator	**Role and Contribution**
Dr. Srinivas Mantha	• **Primary investigator** • Conceived the research idea • Protocol preparation • Will be taking part in every stage of the study taking the responsibilities as primary investigator • Manuscript preparation and modification of various versions during the course of potential publication process
Dr. T. Sudha Lakshmi	• **Co- investigator** • Protocol review • Logistics for the study at Indo-US hospital (site 1) • Screening and enrolment Counselling the subjects in the study at site 2 • Counselling subjects and referral for further evaluation and management on finding of abnormal test (blood or CCIMT) results during the course of study (blood • Review of various manuscript versions during the course of potential publication process
Dr. Dasari Prasada Rao	• **Co- investigator**

Investigator	**Role and Contribution**
	• Protocol review • Logistics for the study at Indo-US hospital (site 1) • Screening and enrolment Counselling the subjects in the study at site 1 • Counselling subjects and referral for further evaluation and management on finding of abnormal test (blood or CCIMT) results during the course of study (blood • Review of various manuscript versions during the course of potential publication process

The following doctors from abroad helped in planning this study and their contribution is gratefully acknowledged. Hence, they will be included as co-authors for the potential publication

Dr. Lee A. Fleisher, MD
Chair, Department of Anesthesiology
and Critical Care,
University of Pennsylvania Health System
Philadelphia, PA 19104

Dr. Michael F. Roizen, MD
Chief wellness officer at the Cleveland Clinic.
Cleveland, Ohio , USA
drmikerealage@gmail.com

10. References

1. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 2007;115:459-67.
2. Kassi E, Adamopoulos C, Basdra EK, Papavassiliou AG. Role of vitamin D in atherosclerosis. Circulation 2013;128:2517-31.
3. Khan A, Dawoud H, Malinski T. Nanomedical studies of the restoration of nitric oxide/peroxynitrite balance in dysfunctional endothelium by 1,25-dihydroxy vitamin D3 - clinical implications for cardiovascular diseases. Int J Nanomedicine 2018;13:455-66.
4. Turan A, Hesler BD, You J, Saager L, Grady M, Komatsu R, Kurz A, Sessler DI. The association of serum vitamin D concentration with serious complications after noncardiac surgery. Anesth Analg 2014;119:603-12.
5. Roizen MF, Roizen JD. Vitamin D and your patients: don't accept wimpy. Anesth Analg 2014;119:503-5.
6. Engelen L, Ferreira I, Stehouwer CD, Boutouyrie P, Laurent S, Reference Values for Arterial Measurements C. Reference intervals for common carotid intima-media thickness measured with echotracking: relation with risk factors. Eur Heart J 2013;34:2368-80.
7. Howard G, Sharrett AR, Heiss G, Evans GW, Chambless LE, Riley WA, Burke GL. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. Stroke 1993;24:1297-304.
8. Stein JH. Carotid intima-media thickness and vascular age: you are only as old as your arteries look. J Am Soc Echocardiogr 2004;17:686-9.
9. Devereaux PJ, Sessler DI. Cardiac Complications in Patients Undergoing Major Noncardiac Surgery. New England Journal of Medicine 2015;373:2258-69.
10. Beattie WS, Wijeysundera DN, Chan MTV, Peyton PJ, et al and Investigators ACTNftE-I. Implication of Major Adverse Postoperative Events and Myocardial Injury on Disability and Survival: A Planned Subanalysis of the ENIGMA-II Trial. Anesth Analg 2018;127:1118-26.

11. Glance LG, Faden E, Dutton RP, Lustik SJ, Li Y, Eaton MP, Dick AW. Impact of the Choice of Risk Model for Identifying Low-risk Patients Using the 2014 American College of Cardiology/American Heart Association Perioperative Guidelines. Anesthesiology 2018;129:889-900.
12. Fleisher LA. Preoperative Cardiac Evaluation before Noncardiac Surgery: Reverend Bayes's Risk Indices and Optimal Variables. Anesthesiology 2018;129:867-8.